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Production of a new diary product enriched with antioxidants from grape pomace and berries

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GOAL OF THE STUDY

The main aim is utilization of the grape pomace, piled up as a waste after winemaking and its application in producing biodiverse and furthermore a novel yoghurt enriched with antioxidants, including polyphenolics from grape pomace and berries. This will improve its nutritional value, contributing to food preservation and significantly reducing the risk of diseases. In fact, the purpose was to introduce a new natural source of biofact and additionally, a new source of antioxidants obtained from pomace of Macedonian grapes for the first time, and engage those compounds (antioxidants) in preparation of a new yoghurt product. If proved to be test – longer and more beneficial for human health than the traditional one, since the antioxidants play a great role in the prevention of cardiovascular disease, cancer etc.

INTRODUCTION

- The largest fraction of winery waste is pomace, that is thrown away and/or up in landfills.
- During the management of these wastes there is production of greenhouse gases (GHG) which must be taken into account. Winery leftovers pile up fast and can cause some serious environmental problems if not disposed of properly [1].
- There is a growing interest by the consumers in foods that are recognized as beneficial for human health because they are either low fat, or contain bioactive materials which reduce the risk of diseases.
- Ideally, these foods should be appealing, taste good, low in price and, most importantly for consumers' acceptability, should contain all-natural ingredients, in a single, easily-accessible product.

MATERIALS & METHODS

➢ **Extraction of polyphenolic compounds from samples**

- about 10 g of each sample (Zupanka, Kardinal, Prokupac, Vranec, Blueberry, Aronia) were measured.
- the extraction was performed twice (50 ml extraction solution: ethanol/water/acetic acid = 80/20/1, V/V/V).
- this mixture was stirred for 30 min by using magnetic stir bar.
- both supernatants brought to a final volume of 200 ml with distilled water.
- all extractions for each grape sample were performed in triplicate.
- the extracts were filtrated, to disregard the solid leftovers of the samples used.

➢ **Determination of total polyphenols**

- the Folin-Ciocalteu method was used for determination of the total amount of polyphenols in each of the 6 samples [2].

➢ **Determination of total anthocyanins**

- the determination of total anthocyanins was realized by the method proposed by Di Stefano et al (1989) [3].


Dry extract from 65 ml of Kardinal

➢ **Concentration of the extracts with rotoevaporation**

- from each phenolic extract (650 ml) different volumes were taken: 10 ml, 50 ml and 100 ml.
- these were vaporized till the formation of dry extracts;
- each dry leftover was reconstructed in 2 ml of distillated water.

➢ **Application of phenolic dry extracts in milk during fermentation**

- 2 l of milk (without any additives) was pasteurized on a temperature of 85-90°C, without getting boiled.
- purchased yoghurt was added as a source of lactic bacteria.
- the milk was separated: 100 ml milk was put into 18 plastic glasses, followed with addition of polyphenolics extract with different concentrations.
- for every sample 3 different rate were obtained (1-10, 1-50 and 1-100) containing different phenolic concentration.


Three different concentrations of phenolic extracts from blueberry before fermentation

➢ **Application of phenolic dry extracts after fermentation i.e. in yoghurt**

- 2 l milk was at first pasteurized at 85-90oC and after cooling at 36-40 °C the lactic bacteria (purchased yoghurt) was added.
- the whole milk was fermented and then, the obtained yoghurt was divided into 18 plastic glasses, followed with addition of polyphenolics extracts with different concentrations obtaining 3 different rate (1-10, 1-50 and 1-100) for each sample.


Fermented milk/Yoghurt enriched with polyphenols added after fermentation

➢ **pH – Measurement of the new yoghurt**

- all yoghurt samples were kept for a period of 1 month at 4 oC and pH measurements were performed at every 2 days during the first 2 weeks, and then, at every 7th day.

RESULTS

➢ **Spectrophotometric Analysis**

Total anthocyanins assay (TA)		Total phenolic assay (TA)	
Name of sample analyzed	TA average value	Name of sample analyzed	TA average value
1 - Zupanka	113	1 - Zupanka	87,500
2 - Kardinal	15.930	2 - Kardinal	76,89
3 - Prokupec	197,69	3 - Prokupec	246,52
4 - Vranec	717,36	4 - Vranec	551,07
5 - Blueberry	238,375	5 - Blueberry	192,315
6 - Aronia	220,86	6 - Aronia	421,300

➢ **Sensory Analysis of the new product**

- this new yoghurt samples had their creamy texture, with no changes in the smell, flavor, no bitterness or astringency in the taste was observed.
- the color was slightly differing, between the samples and the concentration.


Fermented milk without any additives
Yoghurt with polyphenols added during fermentation (Kardinal, Blueberry)

➢ **Microbiological analysis of the obtained yoghurt products**

- to check the validity and the quality of the new yoghurt, the following 4 samples (Zupanka 1 – 100 before fermentation; Vranec 4 – 100 before fermentation; Aronia 6 – 100 before fermentation; Control 1) were tested for Salmonella spp, Staphylococcus aureus, Escherichia coli and counting of colonies of microorganisms; their presence was proved to be negative.


Comparison in the results(the petri dish on the left is yoghurt)

➢ **pH – variation of the obtained yoghurt**

pH values of the control yoghurt without any additives

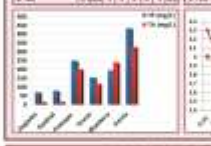
	31.01	02.02	04.02	06.02	09.02	15.02	22.02	02.03
Control	4	3,9	3,8	3,8	3,8	3,8	3,8	3,7

pH values of yoghurt with polyphenols added during fermentation (1-10)

Polyphenols added (Concentration)	31.01	02.02	04.02	06.02	09.02	15.02	22.02	02.03
Zupanka								
1-10	4	4	4	4	3,8	3,8	3,8	3,8
1-50	4,0	4	4	4	3,8	3,8	3,8	3,8
1-100	4,0	4	4	4	3,8	3,8	3,8	3,8
Kardinal								
1-10	4,1	3,8	4	3,8	3,8	3,8	3,8	3,8
1-50	4,1	3,8	4	3,8	3,8	3,8	3,8	3,8
1-100	4,0	3,8	4	3,8	3,8	3,8	3,8	3,8
Prokupec								
1-10	3,8	3,7	3,8	3,8	3,8	3,8	3,8	3,8
1-50	4,1	3,8	4	3,8	3,8	3,8	3,8	3,8
1-100	4,2	4	4,1	4	4	4	3,8	3,8
Vranec								
4-10	4,0	3,8	3,8	3,8	3,8	3,8	3,8	3,8
4-50	4,1	3,8	3,8	3,8	3,8	3,8	3,8	3,8
4-100	4,2	4	3,8	4	4	4	4	4
Blueberry								
5-10	4,0	3,7	3,8	3,8	3,8	3,8	3,8	3,8
5-50	4,1	3,8	4	3,8	3,8	3,8	3,8	3,8
5-100	4,2	4	4	4	4	4	3,8	3,8
Aronia								
6-10	4,0	3,7	3,8	3,8	3,8	3,8	3,8	3,8
6-50	4,1	3,8	4	3,8	4	3,8	3,8	3,8
6-100	4,1	3,8	4	4	4	4	3,8	3,8

pH values of yoghurt with polyphenols added after fermentation

Polyphenols added (Concentration)	31.01	02.02	04.02	06.02	09.02	15.02	22.02	02.03
Zupanka								
1-10	4	3,8	3,7	3,7	3,7	3,8	3,8	3,7
1-50	4	3,8	3,8	3,8	3,8	3,8	3,8	3,7
1-100	4	3,8	3,8	3,8	3,8	3,8	3,8	3,7
Kardinal								
2-10	3,8	3,8	3,8	3,7	3,7	3,8	3,8	3,8
2-50	4	4	3,8	3,8	3,8	3,8	3,8	3,8
2-100	4	4	3,8	3,8	3,8	3,8	3,8	3,8
Prokupec								
1-10	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
1-50	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
1-100	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
Vranec								
4-10	3,8	3,8	3,8	3,8	3,8	3,8	3,8	3,8
4-50	3,8	3,8	3,8	3,8	3,8	3,8	3,8	3,8
4-100	3,8	3,8	3,8	3,8	3,8	3,8	3,8	3,8
Blueberry								
5-10	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
5-50	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
5-100	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
Aronia								
6-10	3,8	3,8	3,8	3,8	3,8	3,8	3,8	3,8
6-50	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8
6-100	4	3,8	3,8	3,8	3,8	3,8	3,8	3,8



DISCUSSION

Three different concentrations of polyphenols were vaporized and applied on a trome – made yoghurt during and after fermentation. As a future work I suggest using other types of grape pomace and applying different solvents for extraction, the polyphenols can be applied on another type of milk and yoghurt culture can be added before fermentation.

CONCLUSION

Prokupec contains the highest amount of both polyphenols and anthocyanins, while Zupanka has the lowest. The yoghurt with polyphenols added during fermentation, presented more stable pH values and the yoghurts with the highest phenolic concentration – 100 ml, showed best results. Zupanka has shown the highest results (pH=4.4 for 1-100) for all concentrations, while Vranec has always the lowest. The new product has an appealing taste, lacking of bitterness and astringency and without any unpleasant taste. This laboratory test of the yoghurts stated that no harmful bacteria are present.

REFERENCES

[1] http://pasteurpress.com/2012/10/winepomace-to-yours-preservatives-and-gluten-dieting-with-winery-waste/
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[3] Di Stefano R., Cravero M. C., Gentilini N. (1989). Metodi per lo studio dei polifenoli dei vini. L'Enotecnico I, Maggio, 83-89.